

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. :	10/080,713	Confirmation No.: 9155
Applicant :	Alan Colman et al.	
Filed :	February 25, 2002	
TC/A.U. :	1600/1632	
Examiner :	Thaia N. Ton	
Title :	Method of Preparing a Somatic Cell for Nuclear Transfer	
Docket No. :	10758.105015	
Customer No. :	20786	

April 21, 2005

DECLARATION OF JORGE A. PIEDRAHITA, Ph.D.

1. My name is Jorge A. Piedrahita. I am Professor of Genomics in the College of Veterinary Medicine at North Carolina State University (NCSU), where my research is focused on the production of transgenic animals by somatic cell nuclear transfer (SCNT) and on the development of methods of homologous recombination in somatic cells.
2. I attended the University of British Columbia, Vancouver, where I obtained a B.Sc. in Agriculture in 1981. In 1984, I obtained a M.Sc. in Animal Sciences from the University of California, Davis, where I was also awarded a Ph.D. in Cell and Developmental Biology in 1998. Following my graduate studies, I was a Postdoctoral Fellow in Molecular Genetics at the University of North Carolina, Chapel Hill.
3. Since completion of my post-doctoral research in 1991, I have spent my career in academia.
4. From 1991-1997, I was an Assistant Professor in the Department of Veterinary Sciences and the Department of Veterinary Anatomy at Texas A&M University (TAMU). In 1997, I was promoted to Associated Professor in both Departments, and appointed Co Director of the Transgenic Core Facility at TAMU. In addition, I was named as a Member of the Intercollegiate Faculty of Genetics.
5. In 1998, to further my interest in SCNT, I spent several weeks working in the laboratory of Dr. Randy Prather at the University of Missouri-Columbia. Dr. Prather is a leading researcher in cloning, and is considered an expert in SCNT.
6. In 1999, I spent six months as a Visiting Scientist/Fogarty Fellow in Hamilton, New Zealand to further my understanding and skill in SCNT.
7. Upon my return from New Zealand, I established the SCNT program at TAMU in 1999.

BEST AVAILABLE COPY

USSN 10/080,713

8. From 1999-2002, I served as Associate Director of the Center for Animal Biotechnology and Genomics at TAMU. During that time, I was also appointed and served as Vice-Chair of the Interdisciplinary Genetics Program and Chair of the Professional Program in Biotechnology.

9. I am presently a Professor of Genomics in the College of Veterinary Medicine of North Carolina State University (NCSU). I also serve as the Interim Director of the Center for Comparative Medicine and Translational Research at NCSU.

10. My research at NCSU is directed to the cloning of transgenic animals by SCNT, and in particular, cloning of transgenic cattle and swine. My research is also directed to the study of methods for homologous recombination in cultured somatic cells.

11. I am the author of over 70 peer-reviewed publications, the inventor of several pending and issued patent applications, and have received numerous awards in the field.

12. I have been given a copy of the following documents: (i) U.S. Patent Application No. 10/080,713 ("Method of Preparing a Somatic Cell for Nuclear Transfer"); (ii) the Office Action dated January 24, 2005; (iii) the Response to Office Action (RCE) dated July 25, 2005; and (iv) the Ayres Declaration and supporting references.

13. I have read and I understand the documents referenced in paragraph 12.

Somatic Cell Donors

14. I understand that this matter involves the state of research in 1999 in the field of nuclear transfer as well as homologous recombination. I was active in the field of nuclear transfer and homologous recombination in 1999. The researchers in these areas at that time, and today, are highly skilled and highly educated. My general observation is that scientists acting independently in these fields in 1999 had a Ph.D. degree and significant work experience in sophisticated laboratory molecular biology techniques. They were among the "elite" of the animal veterinary research profession.

15. I understand that one of the issues raised by the Examiner is whether those of us in the field in 1999 would have thought that it was unpredictable which somatic cells could be used in somatic cell nuclear transfer to produce a clone. My response is that it was understood and well accepted by those of us working independently in the field at the time that the genetic material from any somatic cell could be used in somatic cell nuclear transfer. It was also understood and discussed that the more differentiated the cell, the less efficient the reprogramming might be, however, that was expected and accounted for. It was generally observed that some somatic cells had a higher cloning efficiency than others. However, I, as well as others, distinguished cloning efficiency from cloning ability. Low cloning efficiency simply meant that more transfers were required to achieve a success. In 1999, I knew of no somatic cell that for theoretical or technical reasons could not be used as a supply of genetic material for cloning, or in particular, mammalian cloning.

16. In 1999 I was not aware, and today am still not aware, of any publications or disclosures that reported that a certain somatic cell cannot be used for somatic cell nuclear transfer,

USSN 10/080,713

and in particular mammalian cell SCNT. A number of articles discussed the efficiency of SCNT in 1999, but none that I can recall made a statement that SCNT could not be successfully accomplished with any somatic cell using standard techniques in the industry. Those of us in the field of SCNT live with low efficiency results and expect them in the area of nuclear transfer and cloning. We are typically both time and resource constrained, and therefore use the somatic cells that are known to produce the highest numbers of live offspring simply out of convenience. That, however, should not and can not be interpreted as an implication that genetic material from other somatic cells can not be used.

17. In my own work, I typically use fetal fibroblasts for SCNT. I have also used other somatic cell types including follicular cells, but I generally use fetal fibroblasts simply because they are easy to work with and the goal of my work is to efficiently produce live offspring. I regularly transfer about 400 embryos to achieve a live birth via SCNT even with this highly efficient somatic cell.

18. If in 1999 or today, I were asked to carry out nuclear transfer to achieve a clone using a cell other than a fetal fibroblast, I would transfer far more, e.g., up to 2000 or more embryos, and would consider that a routine part of the lab work. If asked to clone with a difficult or inefficient cell line, I would commit more time and resources to the project as needed to achieve success. The transfer of a larger number of embryos naturally involves more work and cost, but can be carried out by repetition of standard techniques.

19. I have read and I agree with the Declaration of David Ayares. I am of the opinion that Dr. Ayares' Declaration represents the views of those working independently in the field of nuclear transfer in 1999. There is no question that the concept of cloning efficiency is simply a reflection of the ratio of number of attempts and not any indication of the lack of clonability of the cell. Again, there is no good scientific rationale to support the position that any given somatic cell cannot be cloned.

20. I would also like to comment on the Oback and Wells paper. I know David Wells, the senior author on the Oback paper, and as stated above, in 1999, I spent six months as a Visiting Scientist/Fogarty Fellow in Hamilton, New Zealand in the Wells laboratory to further my understanding and skill in SCNT. After I left, Oback worked and trained under the direction of David Wells. Wells does not state in the paper that there are somatic cells that are unclonable. His emphasis is on cloning efficiency. Further, I, as well as others, would know that Table 1 of his paper describes populations of cell experiments that are too small to reach any definitive conclusion on an accurate cloning efficiency of the listed somatic cells. The Oback and Wells paper focuses on differences among donor cells. The factors discussed therein are simply explanations, or rationalizations, of the differences in cloning efficiencies, not ultimate clonability. I further note that in the last sentence of the article (page 162), Wells states that "Cloning from adult neurons presents a considerable challenge and even though initial attempts failed (Wakayama et al, 1998), it cannot be considered biologically impossible". This is consistent with my statements above that there is no good scientific rationale to support the position that any given somatic cell cannot be cloned.

USSN 10/080,711

Homologous Recombination

21. I have been told that that the Examiner has stated that "although homologous recombination may be an essential event in all cells, this does not provide guidance with regard to the targeting of a particular homologous recombination event, and the subsequent selection of a particular cell." In fact, any cell that divides can be used to successfully carry out homologous recombination. A cell need not be highly proliferative, or even moderately proliferative, to undergo targeting by homologous recombination. Rather, homologous recombination requires only that a cell proliferate, regardless of rate.

22. The technique of homologous recombination is described in the standard textbook "Gene Targeting: A Practical Approach. Alexandra L. Joyner, ed. Oxford University Press (1993).

23. I agree with Dr Ayares that it had been well described in the literature in 1999 to obtain and screen for homologous recombination events using PCR and FACS-based screening regardless of whether the genetically modified cells had high proliferative potential. These methods permit a researcher to detect targeted integration events without prolonged in vitro growth and expansion. The methods are also independent of what kind or kinds of cell is transfected. Therefore, it was irrelevant that the Zimmer and Gruss paper mentioned in Paragraph 26 of Dr. Ayares' declaration focused on ES cells or that the Jasin paper mentioned in Paragraph 27 of the Ayares Declaration focused on cultured COS-1 cells. The techniques, FACS and PCR, are equally applicable to all cells.

24. I declare further that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing thereon.

4/25/06
Date


Jorge A. Piedrahita, Ph.D.